

The University of Chicago

TEMPERATURE AS A PREDETER-
MINING FACTOR IN THE DEVELOP-
MENT OF *AVENA SATIVA*

A DISSERTATION SUBMITTED TO THE FACULTY OF THE
DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY
1932

By
THORA MARGGRAFF PLITT

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TEMPERATURE AS A PREDETERMINING FACTOR IN THE DEVELOPMENT OF *AVENA SATIVA*¹

THORA M. PLITT

(WITH THREE FIGURES)

Introduction

It has been shown that various conditions prevailing during the germination of seeds or during the early seedling stages have more or less pronounced effects on the later development of plants. The most comprehensive summary of such investigations is that of KIDD and WEST (14). The effects of different temperatures during the germination period on later plant development have been investigated by a number of workers. APPEL and GASSNER (1) reported that both summer and winter cereals were injured if their germination took place at unfavorably high temperatures. GASSNER (10) brought out the fact that certain types of oats lodged if they were germinated at 25° C. and then transplanted to the field. Lodging occurred even if the 25° temperature prevailed only during the first two days of germination. In either case indications of lodging appeared about five weeks after germination. Under these conditions the oats also failed to head and consequently to yield any grain. The detrimental effects brought about by high temperature were apparently due to disturbances in the very complex chemical reactions in the plant. These findings are confirmed by those of MAXIMOV (19). WALSTER (24), as a result of an investigation on the formative effects of high and low temperatures on the growth of barley, suggested that the course of development of barley is to a large extent predetermined at a very early stage in growth by the chemical equilibria within the seedling, especially the carbohydrate-nitrogen ratio.

The present investigation was undertaken with the purpose of obtaining some indications as to what differences, if any, are produced in the germinating grains and in the seedlings of *Avena* because of the different temperature conditions prevailing during germination.

Part I

Methods

A. GROWING THE PLANTS

Kherson oats, Nebraska 21 selection, kindly furnished by DR. W. E. LYNESS of the Agricultural Experiment Station of the University of Ne-

¹ Contribution from the Hull Botanical Laboratory, University of Chicago.

braska, were used. The Nebraska 21 oats were selected in 1907 from a field of Kherson oats originating from seed imported from Kherson, Russia, in 1896; this selection has since been grown continuously at the Station.

The grains were first treated with a 0.25 per cent. solution of uspulun for about 20 minutes and then placed on porous porcelain plates in covered glass bowls. Water was placed in the bowls to reach part way up the sides of the plates, and more added later as needed. The bowls were then placed in a refrigerator at 5° or in a Minnesota type germinator at 25° C. according to the condition desired. These two temperatures were chosen because they had been used by GASSNER (10) in his investigation of grains.

At the end of five days the entire grains were dried with absorbent paper, weighed, and placed in sufficient hot 95 per cent. alcohol to make a final extract of 80 per cent. alcohol. About 0.5 gm. of calcium carbonate was added to counteract any acidity in the sample. The whole was heated for about 30 minutes on the steam bath to stop enzymatic action, and set aside for a few days. The extract was then poured off and the grains ground in a mortar before reextraction with 80 per cent. alcohol.

In order to investigate the seedlings, the grains were first treated with 0.25 per cent. uspulun for about 20 minutes and then placed on moist cellulocotton in petri dishes. After having been five days in the germinator or the refrigerator, the seedlings were planted in boxes built to fit into glass chambers, about 100 × 90 × 25 cm. These boxes were filled with good garden soil. In these chambers both the temperature and the humidity were controlled. The treatments given the plants are shown in table I.

When the seedlings were three weeks old from the time they were first placed in the refrigerator or the germinator, the tops were cut off at the soil line, separated into two samples, weighed, and treated as previously discussed for the seeds.

The grains in the resting condition were ground in an Enterprise mill, weighed, and extraction carried on as with the seeds. In this and in all

TABLE I
TREATMENT AND GROWING CONDITION OF OATS

LOTS	GERMINATION TEM- PERATURE FOR FIRST 5 DAYS	SUBSEQUENT GROW- ING TEMPERATURE FOR 16 DAYS	PERCENTAGE RELATIVE HUMIDITY DURING THESE 16 DAYS
	°C.	°C.	%
A.....	5	15.0	70
AA.....	25	26.4	70
B.....	25	26.4	40
C.....	25	15.5	70
D.....	5	25.0	70
E.....			

the above cases, the second extract gave no reaction for reducing sugars, so extraction was deemed to be complete.

B. CHEMICAL ANALYSIS

DRY WEIGHT.—The percentage dry weight was obtained by heating the plant material in an oven at 103° C. for 30 minutes to stop enzymatic action, then at about 70° C. in a Cenco electric vacuum oven until constant weight was attained.

SOLUBLE CARBOHYDRATES.—The 80 per cent. extract was prepared for the soluble carbohydrate determinations by the tentative method XII, 37, (a) of the Methods of Analysis (2). Potassium oxalate was used to clear the solution of excess lead acetate as advocated by LOOMIS (16). Inversion was accomplished by the official, first action, method for sucrose XXVII, 22 of the Methods of Analysis (2). The resulting reducing sugars were determined by the KERTESZ variation of the Bertrand method (13). The soluble carbohydrates are calculated as glucose.

HYDROLYZABLE CARBOHYDRATES.—Acid hydrolyzable carbohydrates were determined upon samples taken from the dried and weighed residue left from the alcoholic extract. About 2 gm. of residue were accurately weighed and placed in flasks fitted with reflux condensers. Two hundred cc. of water and 12.5 cc. of concentrated hydrochloric acid were added and the whole boiled gently for three hours. The resulting solution containing the hydrolyzed products was filtered off and the wash water of the residue included with it. This was made up to 500 cc. An aliquot was neutralized with powdered sodium carbonate and the reducing sugars determined by the KERTESZ variation of the Bertrand method (13). The acid hydrolyzable carbohydrates are calculated as glucose.

SOLUBLE NITROGEN.—The soluble nitrogen was determined from the alcoholic extract by the method recommended by PUCHER, LEAVENWORTH, and VICKERY (22) with the variations of T. G. PHILLIPS. These consisted in the use of copper sulphate crystals instead of mercury as a catalytic agent, and the omission of the use of potassium permanganate and of sodium thiosulphate at the completion of the digestion.

INSOLUBLE NITROGEN.—Insoluble nitrogen was determined from the residue remaining from the alcoholic extract by means of the official method of Gunning, II, 22 of the Methods of Analysis (2).

Total nitrogen is obtained by the addition of soluble and insoluble nitrogen. Likewise total carbohydrates are obtained by the addition of soluble and hydrolyzable carbohydrates.

C. MICROCHEMICAL EXAMINATION

The methods used microchemically are as follows:

PECTIN.—Ruthenium red (1:10,000): red stain (8).

STARCH.—Iodine potassium iodide: indigo. Chloral hydrate added to clear the tissues if the starch grains were small (8).

REDUCING SUGARS.—Modified Fehling test of cupric tartrate and 10 per cent. sodium hydroxide, heated at about 40° C. for about two minutes (8).

SILICA.—Phenol crystals heated to dissolve them, then clove oil added to prevent recrystallization: pink sheen on silica deposits. The silica method is given in detail by FROHNMEYER (9).

PROTEINS AND AMINO ACIDS.—Xanthoproteic reaction: yellow-orange (8). Biuret reaction: blue to violet (8). Millon's reagent: red (modified by BENSLEY 4). Ninhydrin: blue. The solution (0.1 gm. ninhydrin to 10 cc. water) is added to the section and left standing for one or two hours. LOEW (15) has reported on the use of this reagent.

ALEURONE GRAINS.—Sections are placed in alcohol-ether for 15 minutes to remove fats, then left in borax carmine for about two hours.

HEMICELLULOSE.—Congo red: red stain in walls (21). Chlorzinc iodide: blue stain in walls (21). Solubility in hot 5 per cent. hydrochloric acid (8). Polarizing microscope: walls anisotropic (21).

Results

A. GERMINATION

Grains germinated at 5° for five days have a markedly higher percentage of dry weight than grains germinated at 25° C. for five days (table III). Their percentage dry weight based on the original weight of the grains is also higher (table III). There is no question as to the greater imbibition of water by the grains and seedlings at the higher temperature, as is evident from table II. The greater amount of water imbibed is probably due to the fact that the seedlings at 25° were in a more advanced stage of development than the seedlings at 5° C. The seedlings at the higher temperature were in fact 2 to 3 cm. high, whereas those at the lower temperature showed the hypocotyls just breaking through.

TABLE II

INCREASE IN FRESH WEIGHT OF GRAINS WHEN GERMINATED AT 5° AND AT 25° C.
FOR 5 DAYS

LOTS	GERMINATION TEMPERATURE	ORIGINAL WEIGHT	WEIGHT AFTER 5 DAYS OF GERMINATION
	°C.	gm.	gm.
G	5	100	178
H	25	100	248

Germination at the lower temperature resulted in a somewhat higher percentage of hydrolyzable carbohydrates remaining in the grains, as is shown in the calculations based on the original weight of the grains before germination (table III). The higher percentage of hydrolyzable carbohydrates is probably due to the fact that starch hydrolysis proceeds more slowly at low temperatures than at high temperatures. Moreover at the higher temperature the more rapid respiration results in a greater consumption of carbohydrates. The total nitrogen does not, of course, differ materially. Thus the proportion of carbohydrates to nitrogen is somewhat higher at the lower germination temperature. The proportion of soluble to insoluble nitrogen is slightly lower under these conditions.

B. MICROCHEMISTRY

There are several differences evident in seedlings germinated at the different temperatures. At low temperatures some starch is deposited as very small grains in the parenchymatous tissues of the coleoptile, the coleorhiza, the scutellum, the cortex of the root, and the root cap. At the higher temperature, however, starch is not deposited in the seedling, or if so, only a little which tends to disappear soon. In the resting grain there is no starch in the embryo.

Another difference is in the amount of pectin produced. At low temperature there is pectin all through the coleoptile and the coleorhiza. It is especially abundant in the epithelium of the scutellum. There is some pectin present in the epiblast and in the root cap. At the higher temperature pectin is present in similar amounts in the coleoptile, the coleorhiza, the epithelium of the scutellum, and the epiblast. But there was a great deal more pectin in the root cap and in addition there was considerable pectin in the epidermis of the root. The seedlings which at this higher temperature had attained a height of about 2 cm. exhibited a great deal of pectin in the cell walls of the coleoptile. Pectin was especially prominent in all of the cell walls of the bundles of the coleoptile except the lignified xylem elements. Reducing sugars could not be detected in the embryo of the resting grain. In the grains germinating at 5° C. reducing sugars could not be detected until the third day; even then there were only traces present which could not be localized satisfactorily. By the fifth day there was not much more in evidence. On the other hand, in grains germinating at 25° C. traces of sugar could be detected after one day. On the second day it was found present in the parenchyma of the root and coleoptile.

The majority of the seedlings germinated for five days at 25° C. were 2 to 3 cm. high, as stated previously. These seedlings were high in reducing sugars, particularly in the cortical parenchyma and the epidermis of the stem; some appeared also in the primary leaves. This confirms the

TABLE III

ANALYSIS OF GRAINS IN THE RESTING CONDITION AND AFTER GERMINATION FOR 5 DAYS AT 5° AND AT 25° C. CALCULATIONS MADE ON BASIS OF DRY WEIGHT (UPPER PART OF TABLE), ON BASIS OF FRESH WEIGHT (MIDDLE PART OF TABLE), AND ON BASIS OF ORIGINAL WEIGHT BEFORE GERMINATION (LOWER PART OF TABLE)

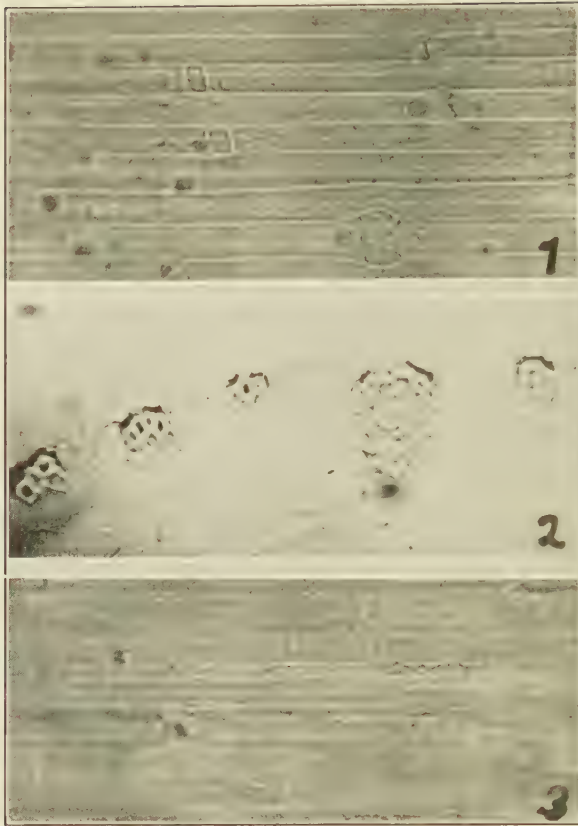
	CONDITION	DRY WEIGHT	SOLUBLE CARBOHYDRATES	HYDROLYZABLE CARBOHYDRATES	TOTAL CARBOHYDRATES	HYDROLYZABLE CH SOLUBLE CH	SOLUBLE NITRO-GEN	INSOLUBLE NITRO-GEN	TOTAL NITRO-GEN	INSOLUBLE N SOLUBLE N	CARBOHYDRATES NITROGEN
		%	%	%	%		%	%	%		
F	Resting	91.9	1.528	60.92	62.45	100	0.071	1.683	1.754	100	36.77
	5° C.					2.5				4.2	
G	5 days	53.39	0.205	54.67	54.88	100	0.101	1.801	1.902	100	28.85
	25° C.					0.4				5.6	
H	5 days	37.86	2.428	52.53	54.96	100	0.120	1.901	2.021	100	27.19
						4.6				7.9	
F	Resting	91.9	1.404	56.00	57.40	100	0.065	1.547	1.612	100	36.43
	5° C.					2.5				4.2	
G	5 days	53.39	0.110	29.19	29.30	100	0.054	0.962	1.016	100	28.73
	25° C.					0.4				5.6	
H	5 days	37.86	0.926	19.89	20.82	100	0.057	0.720	0.777	100	26.79
						4.6				7.9	
G	5° C.	95.04	0.195	51.96	52.16	100	0.096	1.712	1.808	100	28.84
	5 days					0.4				5.6	
H	25° C.	90.86	2.207	47.73	49.94	100	0.137	1.727	1.864	100	26.79
	5 days					4.6				7.9	

macrochemical findings of a considerably higher percentage of soluble sugars when germinated at 25° than when germinated at 5° C. (table III).

The protein and amino acid tests, xanthoproteic, biuret, Millon, and ninhydrin, indicated a more rapid mobilization of these substances when germination took place at 25° than at 5° C. In the resting grain all these stains were positive in the embryo and the aleurone layer. The layer of large cells immediately below the aleurone layer was stained particularly with Millon's reagent. In the aleurone layer there are present many aleurone grains (borax carmine method); in these aleurone grains there are no inclusions. During germination at the lower temperature the growing point, the primary leaves, and the root gradually take deeper stains. The ninhydrin test shows the most change. Whereas in the resting grain only a faint blue rim of color is obtained about the edge of the cover glass, as germination proceeds the color becomes deeper and localized in the regions just mentioned; moreover this reagent seems to stain the vascular tissues particularly. At the higher germination temperature after one day the primary leaves and the root stand out. After two days the biuret reaction is purple in the growing point and the primary leaves, but bluish in the coleoptile and the root (especially the stele), possibly indicating differences in the protein units present. After three days in the germinator ninhydrin yields a deep blue color in the root tip, the stele of the root, the primary leaves, particularly in the vascular bundles, and in the bundles of the coleoptile. These reactions continue through the germination period.

It was noted that in the resting grain the walls of practically all parenchyma cells are irregularly thickened, particularly at the corners. These thickenings are stained blue by chlorzinc iodide and red by Congo red. They are soluble when the sections were heated to the boiling point in 5 per cent. hydrochloric acid. Examination of the sections with the polarizing microscope before treating with acid showed these thickenings to be anisotropic; but after the acid treatment this quality was lost. As growth proceeds these thickenings decrease slightly in the original regions but appear to become more prominent in the parenchyma of the leaves. This may be an instance of hemicellulose cell wall thickenings, possibly functioning as a reserve food.

Some 6-weeks old plants were examined for the distribution of silica. These plants had been germinated at 5° or at 25° C. for five days and had then been transplanted to a plot of soil in the greenhouse. Of the plants that were germinated at 5° C. individuals were examined which stood erect. The silica was deposited mainly in the walls of the short epidermal cells of the stem (fig. 1). There was also some deposition in the cell walls of the stomata. The irregular occurrence of circular dark spots in the walls of the long epidermal cells of the stem also represent silica deposited in the



FIGS. 1-3. Fig. 1, epidermis of stem of 6-weeks old oat plants showing silicon deposit in cell walls of short cells particularly, some in stomatal cell walls, and traces in circular areas in long cells. Fig. 2, cross section of a leaf sheath of a 6-weeks old oat plant with silicon deposited in certain epidermal cells over the bundles. Fig. 3, epidermis of a leaf sheath of a 6-weeks old oat plant with silicon deposited in separate cells arranged in rows.

cell walls as a fine network. Figure 2 shows the cross section of a leaf sheath, and figure 3 the longitudinal view. It will be noted that the silica is deposited in epidermal cells over the bundles, but that these cells as seen in top view are separated from one another by cells whose walls do not contain silica. A few of those plants which were germinated at 25° C. had lodged. These were examined for silica, but no differences were found between them and the erect plants.

C. AFTER THREE WEEKS

The two series in the controlled chambers were run three weeks apart, but in immediate succession: May 5 to 26 and May 24 to June 15, 1931.

TABLE IV

ANALYSIS OF PLANTS 3 WEEKS OLD GROWN AT 15.5° C. (FIRST AND THIRD PARTS OF TABLE) AND AT 25°-26.4° C. (SECOND AND FOURTH PARTS OF TABLE). UPPER HALF OF TABLE CALCULATED ON DRY WEIGHT BASIS; LOWER HALF ON FRESH WEIGHT BASIS

	GROW- ING TEM- PERA- TURE	RELA- TIVE HUMID- ITY	GERMI- NATION TEM- PERA- TURE	DATES	DRY WEIGHT	SOLU- BLE CAR- BOHY- DRATES	HYDRO- LYZ- ABLE CAR- BOHY- DRATES	TOTAL CAR- BOHY- DRATES	HYDROLYZABLE CH SOLUBLE CH	SOLU- BLE N	INSO- LUBLE N	TOTAL N	INSOLUBLE N SOLUBLE N	CARBOHYDRATES NITROGEN	HEIGHT
	° C.	%	° C.		%	%	%	%		%	%	%			cm.
A	15.5	70	5	5/5-5/26	10.55	2.65	9.09	11.74	100 29.1	0.846	5.591	6.437	100 1.5	1.83	11.4
AA	15.5	70	5	5/24-6/15	11.36	6.00	12.10	18.10	49.9	0.917	5.450	6.367	100 16.8	2.84	11.6
D	15.5	70	25	5/24-6/15	12.12	14.60	18.57	33.17	78.6	1.464	8.682	10.146	100 16.9	3.28	11.8
B	26.4	70	25	5/5-5/26	12.36	8.33	12.14	20.47	100 68.6	0.760	4.800	5.560	100 15.8	3.68	19.6
E	25	70	5	5/24-6/15	11.91	5.61	12.37	17.99	45.4	1.115	4.363	5.478	100 25.6	3.78	18
A	15.5	70	5	5/5-5/26	10.55	0.279	0.959	1.238	100 29.1	0.090	0.611	0.701	100 15.1	1.78	11.4
AA	15.5	70	5	5/24-6/15	11.36	0.676	1.375	2.051	49.9	0.105	0.619	0.724	100 16.8	2.66	11.6
D	15.5	70	25	5/24-6/15	12.12	1.769	2.250	4.019	78.6	0.198	1.052	1.250	100 16.9	3.23	11.8
B	26.4	70	25	5/5-5/26	12.36	1.030	1.501	2.531	100 68.6	0.094	0.594	0.688	100 15.8	3.68	19.6
E	25	70	5	5/24-6/15	11.91	0.668	1.475	2.143	45.3	0.133	0.520	0.653	100 25.6	3.28	18.2

The increased day length in the second period increased all of the carbohydrate fractions, and did not cause any appreciable change in the nitrogen fractions (table IV, A and AA). Notwithstanding this increase in carbohydrates the two series are comparable, for the results show the same trend. Thus the proportion of carbohydrates to nitrogen is less in both A and AA, germinated at low temperature, than in B or D, germinated at the higher temperature.

At the end of three weeks the seedlings grown at about 25° (table IV, B and E) were the taller regardless of the other conditions applied in these experiments. Seedlings germinated at 25° (table IV, B and D as compared with AA and E) exhibit a higher percentage of dry matter than those germinated at 5° C., also higher percentages of soluble carbohydrates and generally a higher percentage of total carbohydrates. There is a decidedly higher proportion of soluble to hydrolyzable carbohydrates: 68.6:100 and 78.6:100 (B, D) as compared with 49.9:100 and 45.4:100 (AA, E).

Those plants which were germinated at 25° C. and grown at about the same temperature (table V, B) were higher in all the carbohydrate fractions and slightly lower in all the nitrogen fractions than those germinated at 5° and grown at 15° C. (table V, A). The total carbohydrates are 8 per cent. higher and total nitrogen is about 0.9 per cent. lower; hence their proportion of carbohydrates to nitrogen is decidedly higher. The proportion of soluble to hydrolyzable carbohydrates is also far higher than with the other treatment; 68.6:100 (B) at about 25° C. compared with 29.1:100 at low germination and growth temperatures (A).

At this age (three weeks) the proportions of soluble to insoluble nitrogen did not seem to be correlated with any specific factor. For instance, the proportion of insoluble to soluble nitrogen is 100:25.6 when germination took place at 5° and growth at 25° C. (table V, E); but another lot (AA) germinated at 5° and grown at 15.5° C. has a proportion of 100:16.8, and yet another lot (B) germinated at 25° and grown at 25° C. has a proportion of 100:15.8.

One lot of oats was germinated at 25° C. and grown at the same temperature but at a relative humidity of 40 per cent. (table VI, C) instead of 70 per cent. (B). These plants did not produce so rank a growth as the plants grown at a humidity of 70 per cent., as may be seen from the measurements of their heights (table VI); and their percentage of dry matter was slightly higher. The proportion of soluble carbohydrates to hydrolyzable carbohydrates is slightly less. Since the percentage of total carbohydrates is less but the percentage of nitrogen about the same, the ratio of carbohydrates to nitrogen is slightly less when the humidity is decreased.

Discussion

The greater imbibition of water and the greater growth of the seedlings at the end of the 5-day germination period with a temperature of 25° as compared with the behavior of seedlings grown for the same length of time at 5° C. is in agreement with the general findings as summed up by BARTON-WRIGHT (3). He states that not only does the temperature affect the rate of entry of water into the seed as well as the growth rate of the radicle, but it also affects the resistance of the seed coat to the extrusion of the radicle. *Phaseolus vulgaris* shows evidence of germination at 9° C., and the growth rate increases with rising temperatures to 36° and ceases at 46° C. Minimum, optimum, and maximum germination temperatures for rye are 1°, 25°, and 36° C. respectively. The optimum points are somewhat dependent on the time factor.

The results obtained concerning the amount of carbohydrates present in the low temperature seedlings are in agreement with the findings of DICKSON, ECKERSON, and LINK (7) that 10-day old wheat seedlings grown at low soil temperatures are practically high carbohydrate plants.

It is well known that plants respire more rapidly at higher temperatures than at lower ones. This fact is reflected in the percentage of dry weights obtained at the end of the first five days of germination at different temperatures. DAY (6), in working with barley, measured the carbon dioxide output for ten days at three different temperatures. He found that the average output per hour was greater at 60° F. than at 38°–43°, and still greater at 70°. Another feature was that the respiration increases to a maximum and then decreases again. At the lowest temperature given, this maximum occurs on the fourth and fifth days. But at the highest temperature it is shifted to the third day.

It seems likely that the greater amount of soluble sugar present in oat grains germinated for five days at 25° C. is associated with the fact that no starch is deposited in the embryo under those circumstances, whereas starch is deposited at the lower temperatures usually considered more favorable for the growth and development of this plant. In this respect the behavior of oats is similar to that of barley as reported by BROWN and MORRIS (5).

Increase in pectin materials at high germination temperature in oats parallels the results obtained in wheat (7).

Lower germination temperatures are generally recommended as being more favorable for growth and development than higher ones. The different rates of hydrolysis of storage materials and of synthesis of new materials at higher temperatures as compared with lower ones may conceivably affect the later development of plants, directly or indirectly. Thus at the lower temperature the starch in the endosperm is hydrolyzed more rapidly than the protein; also there is less vegetative growth than at the higher

TABLE V
ANALYSIS OF PLANTS 3 WEEKS OLD GERMINATED AT 5° (UPPER PORTION OF TABLE) AND AT 25° C. (LOWER PORTION OF TABLE); CALCULATED ON DRY WEIGHT BASIS

GERMI- NATION TEM- PERA- TURE	GROW- ING TEM- PERA- TURE	RELA- TIVE HUMID- ITY	DATES	DRY WEIGHT	SOLU- BLE CAR- BOHY- DRATES	HYDRO- LYZ- ABLE CAR- BOHY- DRATES	TOTAL CAR- BOHY- DRATES	HYDROLYZABLE CH SOLUBLE CH	SOLU- BLE NITRO- GEN	IN- SOLU- BLE NITRO- GEN	TOTAL NITRO- GEN	IN SOLUBLE N SOLUBLE N	CARBOHYDRATES NITROGEN	HEIGHT
°C.	°C.	%		%	%	%	%		%	%	%			cm.
A	5	15.5	5/5 5/26	10.55	2.65	9.09	11.74	100 29.1	0.846	5.591	6.437	100 15.1	1.83	11.4
AA	5	15.5	5/24 6/15	11.36	6.00	12.10	18.10	100 49.9	0.917	5.450	6.367	100 16.8	2.84	11.6
E	5	25.0	5/24 6/15	11.91	5.61	12.37	17.99	100 45.4	1.115	4.363	5.478	100 25.6	3.78	18.2
B	25	26.4	5/5 5/26	12.36	8.33	12.14	20.47	100 68.6	0.760	4.800	5.560	100 15.8	3.68	19.6
D	25	15.5	5/24 6/15	12.12	14.60	18.57	33.17	100 78.6	1.404	8.682	10.146	100 16.9	3.29	11.8

TABLE VI

ANALYSIS OF PLANTS 3 WEEKS OLD, GERMINATED AT 25°, GROWN AT 26.4° C., BUT AT DIFFERENT RELATIVE HUMIDITIES: 40 AND 70 PER CENT. CALCULATED ON BASIS OF DRY WEIGHTS (UPPER PART OF TABLE) AND ON FRESH WEIGHT BASIS (LOWER PART OF TABLE)

	RELATIVE HUMIDITY	GROWING TEMPERATURE	GERMINATION TEMPERATURE	DATES	DRY WEIGHT	SOLUBLE CARBOHYDRATES	HYDROLYZABLE CARBOHYDRATES	TOTAL CARBOHYDRATES	HYDROLYZABLE CH SOLUBLE CH	SOLUBLE NITROGEN	IN-SOLUBLE NITROGEN	TOTAL NITROGEN	INSOLUBLE N SOLUBLE N	CARBOHYDRATES NITROGEN	HEIGHT
	%	°C.	%		%	%	%	%	%	%	%	%			cm.
B	70	26.4	25	5/5 5/26	12.36	8.33	12.14	20.47	100 68.6	0.760	4.800	5.560	100 15.8	3.68	19.6
C	40	26.4	25	5/5 5/26	13.74	5.73	10.67	16.40	100 53.7	0.840	4.629	5.469	100 18.1	3.00	14.1
B	70	26.4	25	5/5 5/26	12.36	1.030	1.501	2.531	100 68.6	0.094	0.594	0.688	100 15.8	3.68	19.6
C	40	26.4	25	5/5 5/26	13.74	0.787	1.466	2.253	100 53.7	0.118	0.633	0.751	100 18.1	3.01	14.1

temperature (7). In the present investigation both the macrochemical and the microchemical data seem to indicate that the rates of carbohydrate and protein hydrolyses and utilization are definitely affected by an increase in germination temperature. ZALESKI (26) reports that as the protein in *Lupinus angustifolius* is broken down, much asparagin appears. The amount of asparagin obtained increases with rise in temperature. The temperature coefficient follows the VAN'T HOFF rule, $Q_{10} = 2.5$. The indications are that the slower rate of hydrolysis of protein at lower temperatures in oats makes for conditions of growth more favorable to the complete development of the plant.

The most outstanding characteristic of the 3-weeks old plants which had been germinated at 25° C., regardless of the subsequent growing conditions here considered, was the marked increase of total carbohydrates in the tops of the plants over those germinated at 5° C. These high temperature plants had already reached the stage at which photosynthetic activity might begin, at the time the others were just breaking through the seed coats. GREGORY (12) found in *Cucumis sativus* that the growth rate of leaf surface increases with the temperature, and that it is dependent on the area of the leaf surface already present. But on investigating the relative rate of increase (rate of increase per unit of leaf surface already present) he found it to be independent of the temperature. He states that the differences in the final leaf area between plants grown at different temperatures must therefore be related to the time elapsing from germination before the photochemical process can begin, i.e., the time elapsing before expansion of the foliage leaves begins. This is a matter of growth and differentiation in the apex and has what he calls a normal temperature coefficient, $Q_{10} = 2.5$. Thus the delay in the development of the first leaf is determined by the temperature at which the developmental processes occur. He believed that a nitrogenous leaf-forming substance is involved. Thus any later differences in leaf surface are caused by the speed with which the plant attained its first leaf. The present experiment seems to point to the conclusion that, since the seedlings at the higher temperature were further developed when transplanted from the 25° germinator to the chambers than those transplanted from the 5° C. refrigerator, they had the advantage of an earlier start in photosynthetic activity which expressed itself in a higher percentage of carbohydrates in the tops of the plants at the age of three weeks.

Analyses of older plants, such as those of clover by TOTTINGHAM (23), indicate a higher percentage of polysaccharides in plants grown at 16.9° to 23.3° C. than in those grown at 23.2° to 28.2° C. It may be a complicating factor that in this experiment he collected the plants grown at the higher temperature two weeks sooner than the others, in order that all might have

attained the same size for analysis. Yet he again obtained in buckwheat grown at 16.0 to 19° C. a percentage of polysaccharides higher than when grown at 20.5° to 25° C., with all of them harvested at the end of ten weeks. He attributes the decrease mainly to the impossibility of polysaccharide storage at higher temperatures because of greater consumption of sugars with increased respiration. On the other hand it is probable that different optima exist for different stages of development of a plant. GASSNER (10) recommends for Uruguay oats a low temperature germination followed by a rapid rise in temperature in order to achieve maturity as early as possible.

Furthermore McLEAN (20), in investigating effects of climatic conditions, using soy beans, came to the conclusion that temperature was the limiting condition for growth during the first two weeks in practically all cases. During the second two weeks of growth, however, with exactly the same environmental conditions, the moisture relation (rainfall-evaporation ratio) appears to have been the limiting condition, especially if the temperature was high. This must be due to a difference in the internal conditions of the plants at the different developmental stages. Growth during the early period consisted largely in stem elongation, which must have been accomplished at the expense of material stored in the seed. The rate of development of the plants was therefore probably dependent on the rate of hydrolysis of storage materials and of translocation from the cotyledons to the growing points, and hence dependent on the temperature. On the other hand, during the second two weeks while leaf expansion occurs, greater transpiration will increase the water requirement. So the moisture relation would be a greater factor than before.

APPEL and GASSNER (1) report that various cereals, such as wheat, barley, and oats, raised entirely in the warm greenhouse germinated rapidly and continued to grow rapidly. They had already attained a height of 15 cm. when those in the cool greenhouse germinated. After three weeks, however, the rate of growth of those at the high temperature decreased so that finally they were overtaken by those at low temperature. WALSTER (24) noted a similar phenomenon. Barley plants in the warm house, which were several inches high before the plants in the cool house had come up, during the first two weeks maintained a more rapid growth. But at the end of a month the plants in the cool house had outstripped those in the warm house in their growth rate. At the end of six weeks all of the plants in the cool house had outgrown those in the warm house.

Analyses of plants at later stages than those made in this investigation may well yield interesting results.

The findings concerning the highly localized deposition of silica agree with those made by WYSSLING (25) in his survey of the distribution of silica in plants. Since the deposition of silica does not result in a continu-

ous structure, and since silica is only a very small part of the mechanical structure of the stem and leaf sheaths, it seems unlikely that it should be a factor in determining an erect or a recumbent habit of the plant. Rather, as set forth by WYSSLING, it seems to be an excretion of a nonessential element absorbed in different quantities under differing conditions.

Part II

Other varieties of oats were tested for possible responses to different germination temperatures. Through the courtesy of the Agricultural Experiment Station at the University of Tennessee four varieties were obtained, Hatchett, Lee, Turf B, the Fulghum 699-2011. From the State College of Agriculture at the University of Georgia were obtained three other varieties, Fulghum, Norton, and Appler. Each of these varieties was subjected to the following four germination conditions: (a) 2° for 5 days; (b) 25° for 5 days; (c) 2° for 2 days, then 25° for 3 days; (d) 25° for 2 days, then 2° for 3 days. Treatment was begun on May 27, 1932. At the close of the 5-day period the germinating grains or seedlings were planted in a plot of soil in the greenhouse. On September 19 the experiment was terminated.

Observations were made on dates of heading and possible evidences of lodging. In this series there appeared to be no clear correlation between the temperatures prevailing during germination and dates of heading (these dates were late in the season for oats). Dates of heading differed within a single variety, but no general trend was observable when comparing the different varieties. As for evidences of lodging, there was no bending of the culms in the lower nodes or internodes. In a few cases the culms were leaning over or even broken, but this appeared to be the result of the method of watering. At times the temperature of the greenhouse rose considerably, even reaching 124° F. (51.1° C.). It seems obvious that these varieties of oats do not give a lodging response to high temperatures applied either early or late in the life of the plants.

Part III

In order to approximate more closely the experimental material reported by GASSNER (10), some South American varieties of oats were tested. These were secured from Dr. ALBERTO BOERGER, Director of the Agricultural Experiment Station at Montevideo through the courtesy of Mr. JOSE RICHLING, Consul General of Uruguay in New York. These oats are classified as follows: 1095a belongs botanically to the species *Avena byzantina*; BID belongs to the species *A. sativa*, originating from a hybrid of two forms of this species; 64s, *A. capa*, represents a type native of Uruguay and belongs to the species *A. sativa*. GASSNER reports using "*Avena del pais*"

or native Uruguay oats. The seeds falling to the ground in the field make pastures which are good for several years. BOERGER states that although none of these samples was harvested in fallow land, it would be perfectly feasible to obtain them from such land owing to the fact that fallen seeds of any kind soon become transformed into what are called "avena gaucha" (wild oats) offering valuable pasture for winter and fall seasons.

The investigation of the response of these oats to germination temperatures was undertaken in the laboratories of the Botany Department at Columbia University through the courtesy of Professor SAM F. TRELEASE. The oats were treated as previously, namely, germinated at 25° and 5° C. for five days and then planted in the greenhouse. Treatment of the grains was begun on March 25, 1933, and the plants were harvested on June 8.

No lodging was observed in any of these plants.

One lot of the oats headed, namely, the 64s oats which were germinated at low temperature (5° C.). All were bearing grain when harvested. A parallel series, but with germination taking place at 25° C., bore no grain whatsoever. Of the BID oats (5° C. germination) only one culm headed; of the 1095a oats none headed. The varieties are evidently quite distinct from one another, for BOERGER reports the increased yield of the 64s oats. There are also differences in the vegetative portions of the plants: 1095a produces thin culms and fine leaves, BID thicker culms and broader leaves, and 64s the sturdiest plants.

Such a difference in yield was reported by GASSNER (10) when working in Uruguay: Uruguay oats kept for the first ten days at 6°–9° C. headed after two months, likewise those kept for ten days at 6°–9° then for two days at 25° C., and also those kept for the first five days at 6°–9° and then for two days at 25° C. But oats germinated at a constant temperature of 25° C. failed to head by the end of eight months; even those which were exposed to a temperature of 25° only the first two days and then at 6°–9° C. for eight days failed to head.

GASSNER (11) further differentiates between summer and winter cereals. According to him the latter require a certain period of cold at some time in the life cycle in order eventually to go over into the reproductive phase. The summer cereals, on the other hand, will go over into the reproductive phase even without undergoing such a cold period, although they also appear to be influenced by such an exposure. MAXIMOV (19), in testing *Avena byzantina*, found that as the germination temperature was decreased from 26° to 0° C. the vegetative period was shortened from 80 days to 31 days, and simultaneously the yield was increased. The principle of shortening the length of the vegetative stage of grains seems to be applied on a large scale in Russia under the name of Jarovization. The methods em-

ployed and a consideration of the factors involved are presented by LYSSENKO (17, 18).

It was thought that higher germination temperatures than 25° C. might cause greater disturbances in the growth of the oat plants. From June 20 to July 24 a number of plantings were made in the greenhouse at the University of Chicago; these were observed until November 24, 1933. The oats were: Kherson oats Nebraska 21 selection, and the oats 1095a, 64s, and BID from Uruguay. The grains were first germinated for five days at 5°, 10°, 25°, 30°, 32.5°, 35°, 37.5°, and 40° C., then planted in soil plots.

The last two temperatures, 37.5° and 40° C., were evidently fatal, for none of the grains placed in these germinators ever germinated. At 35° C. there was a low percentage germination and subsequent growth was limited. The other stands were fairly good; in no case, however, was any lodging observed.

Only one variety headed, namely, the Kherson oats (Nebraska 21 selection). This is very possibly due to the late date of seeding, for during the spring certain of the 64s oats did head. But of these Nebraska 21 oats only those germinated at 10° and 5° C. headed. These were placed in the refrigerators on June 20 and 26 respectively, and they began to head on August 22 and 29. In this respect these oats were similar to the 64s oats in the preceding series. In no case was any lodging observed. All through the growing period many of the plants were stooling, some of the plants having forty or more culms of all sizes at the end of the experiment. The factor operating to produce this unusual result was not determined.

Summary

1. Oat grains germinated at 25° C. for five days have a markedly lower percentage of dry weight than grains germinated at 5° C. when calculated either on the basis of fresh weight or on the basis of the original weight before germination. These differences are directly related to the rate of growth of the seedlings at the two temperatures, and the amount of carbon and hydrogen consumed in respiration. A somewhat lower percentage of starch remains in the grains when germinated at the higher temperature, and more water is imbibed by the grains than at the lower temperature. The proportion of soluble to insoluble nitrogen is slightly higher at the higher germination temperature.

2. Microchemically it was found that whereas there is no starch in the embryo of oat grains in the resting condition, starch is deposited in the embryo during germination at 5° C.; but little or none is deposited in the embryos germinated at 25° C. At the higher germination temperature there are more pectin and reducing sugars in the seedling than at the lower temperatures.

3. Protein reactions (microchemical) became more intense as germination proceeded, gradually in the case of the lower temperature, much more rapidly at the higher temperature.

4. Cell wall thickenings were found in the parenchyma of the embryo and the early seedling stages of the oats, giving reactions indicating the presence of hemicellulose.

5. Seedlings grown at about 25° C. were the tallest in the series regardless of the germination temperature; they also had the lowest percentages of nitrogen.

6. Seedlings which grew from grains germinated at 25° C. regardless of the later conditions applied in these series exhibited a higher percentage of dry matter than those germinated at 5° C., also higher percentages of carbohydrates, and the nitrogen percentages were lower. There is a higher proportion of soluble to insoluble acid hydrolyzable carbohydrates.

7. Plants germinated and grown at about 25° C. were higher in carbohydrates and slightly lower in nitrogen; also the proportion of soluble to insoluble acid hydrolyzable carbohydrates is far higher than in plants germinated at 5° and grown at 15° C.

8. The proportions of soluble to insoluble nitrogen varied.

9. Oats germinated and grown at 25° C. but at a relative humidity of 40 per cent. as compared with those at 70 per cent. made less growth; their percentage of dry matter was slightly higher, and the percentages of carbohydrates were lower.

10. The greater amount of carbohydrates in plants germinated at 25° as compared with that in plants germinated at 5° C. is believed to be caused by the fact that the seedling development is much further advanced at the time of transplanting, so that photosynthetic activity begins several days earlier.

11. Silica in 6-weeks old plants was found to be deposited in the same manner in both erect and recumbent plants. It is highly localized in certain cells and these cells form no consecutive structure. Hence silica is believed to be a negligible factor in the supporting mechanism of the plant.

12. High temperatures during the very early phases of germination of oats had much less effect on lodging of oats than the work of GASSNER indicates. No temperature treatment during early germination was found that would consistently cause lodging to develop, either immediately or in later life.

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